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Penetration of Cells by Asbestos Fibers

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Studies on the behavior of asbestos fibers within tissues have shown that the only cells that regularly contain asbestos are macrophages and their derivatives. However, these cells actively incorporate the asbestos fibers by the process of phagocytosis, and there is little evidence of direct penetration. Examination of the gut lining after prolonged asbestos ingestion has shown no evidence of dust penetration either through or between the epithelial cells. The structure and arrangement of these cells is discussed, and it is suggested that they are exceptionally well adapted to prevent penetration by any solid material.

Early studies on the biological effects of asbestos centered on the production of asbestosis, bronchial carcinomas, and more recently mesotheliomas. There is now evidence, however, that asbestos dust may penetrate to many different parts of the body *(1,2)*, and it has recently been suggested that heavy asbestos exposure may lead to both laryngeal carcinoma *(3)* and carcinoma of the gastrointestinal tract *(4,5)*. The means by which the asbestos fibers may penetrate to these sites is therefore of great interest and needs to be considered at the electron microscope level.

A survey of the literature for the past ten years would indicate that it is extremely rare for asbestos fibers to be found in any cells other than phagocytes. After heavy lung dusting in experimental animals *(6)*, an occasional small fiber has been seen in the alveolar epithelial cells, and after massive intrapleural injection a few particles may be seen in mesothelial cells *(7)*. Since, however, macrophages abound in both these areas after dust administration, and since all macrophages present quickly become loaded with many dust particles, these two studies merely emphasize how difficult it must be for asbestos to penetrate cells by direct mechanical action. It would appear that cell membranes are much more resistant to the penetration of sharp mineral fibers than might be expected, and in this connection it may be emphasized that phagocytosis is accomplished by a process that results in the deposition of foreign material within the cell cytoplasm without this material ever having to cross the cell membrane. During phagocytosis macrophages form elongated leaflike processes on their surfaces, and these surround foreign material before folding back on to the main cell body. The edges of the processes then fuse with the cell surface, and the phagocytosed material is enclosed within a vacuole that is surrounded by what is basically surface membrane (Fig.1). Initially the phagocytic vacuole or phagosome may be quite large, but eventually this contracts, and the contents become condensed until small spherical structures are formed that are known as phagosome residual bodies (Fig. 2). These still contain mineral particles and debris from other phagocytosed material.

Recent publications have indicated that people exposed to heavy doses of asbestos may have some increased risk of developing neoplasms of the gastrointestinal tract. The ways in which ingested asbestos fibers may react with the gut lining cells have therefore become of great interest. In particular, it is important to deter-

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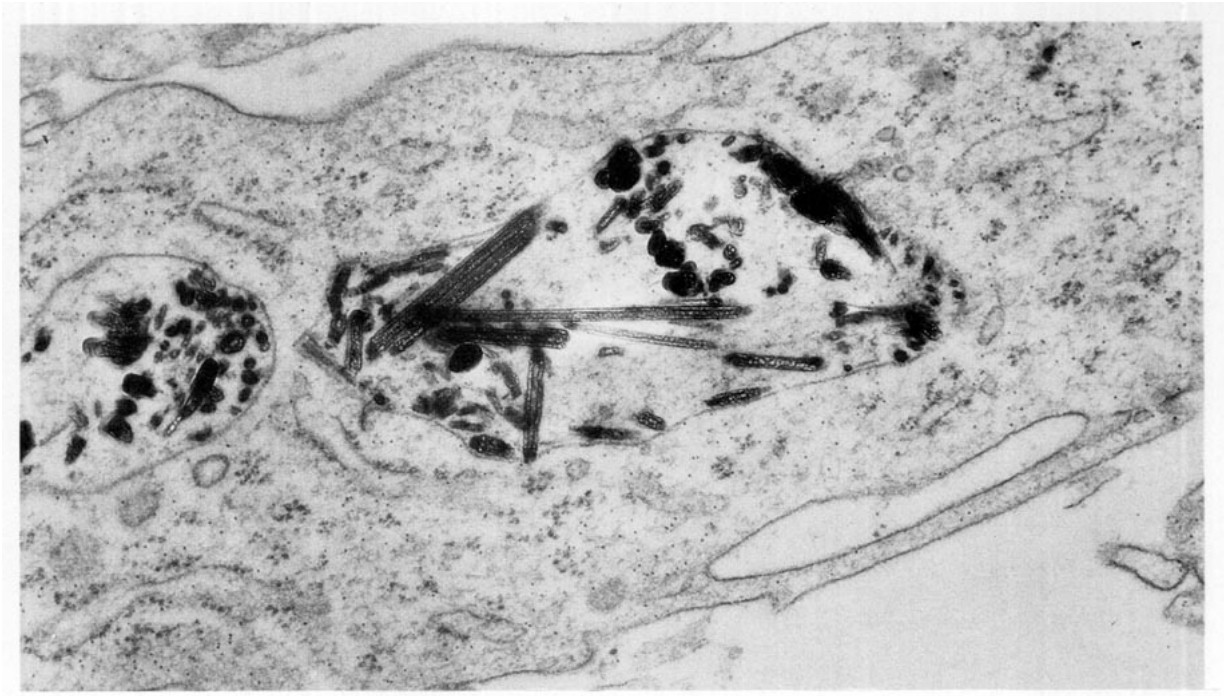

FIGURE 1. Section of a macrophage from a mouse granuloma 2 weeks after the intrapleural injection of chrysotile asbestos heated to 600°C. Groups of chrysotile crystals are seen in two phagosomes within the cell. 46,800×.

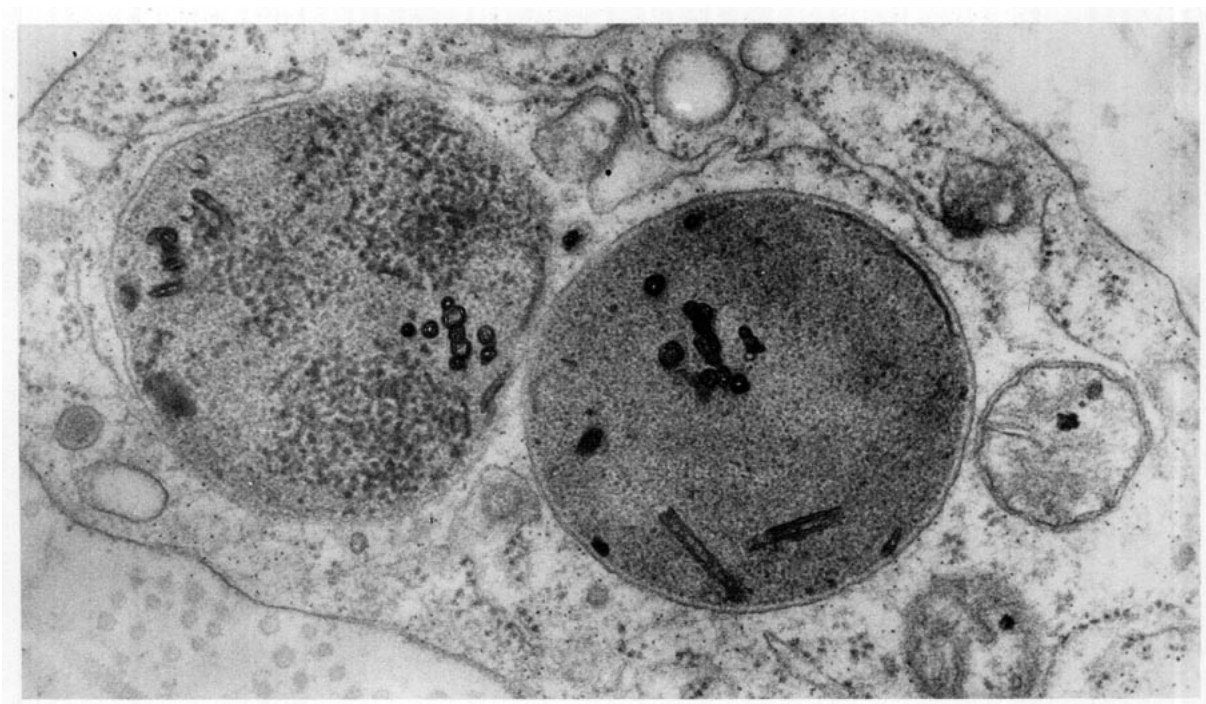

FIGURE 2. Area of macrophage cytoplasm from guinea pig lung following 6 months dusting with chrysotile asbestos. Within the cytoplasm there are two large phagosome residual bodies each containing crystals of chrysotile. 46,800×.

mine whether asbestos fibers present in food can penetrate into the epithelium and perhaps further into the subepithelial connective tissue. In the past, several studies have been undertaken in which rats have been fed large doses of asbestos for long periods. These studies showed neither dust penetration by light microscope examination, nor did the animals develop neoplasms. Since these results were completely negative, however, publications did not appear at the time, but because of recent interest in this subject a compendium paper is planned for the near future. Because tissue examination during these experiments had been limited to light microscopy, the presence of very small fibers would not have been detected.

We have, therefore, commenced a new series of animal feeding studies in which electron microscopy is being used to examine the effect of ingested asbestos fibers on the gut lining. In these experiments rats are fed with asbestos mixed with butter, 5 mg of asbestos/g of butter. The animals are fed the butter mixture *ad libitum* and are also provided with their normal diet of food pellets. On the average, each rat consumes about 20 g of butter each week and therefore ingests approximately 100 mg of asbestos. So far the study has been in progress for 6 months, but tissue examination with the electron microscope has not progressed beyond animals killed after 3 months feeding.

These studies have involved the examination of large numbers of tissue blocks from the stomach, small intestine, colon and mesenteric lymph nodes of rats fed either chrysotile (UICC sample A) or crocidolite (UICC sample). All the cells examined have appeared normal, and no sign of asbestos penetration has been seen (Figs. 3 and 4). It would appear, therefore, that as in other tissues, asbestos fibers have difficulty in

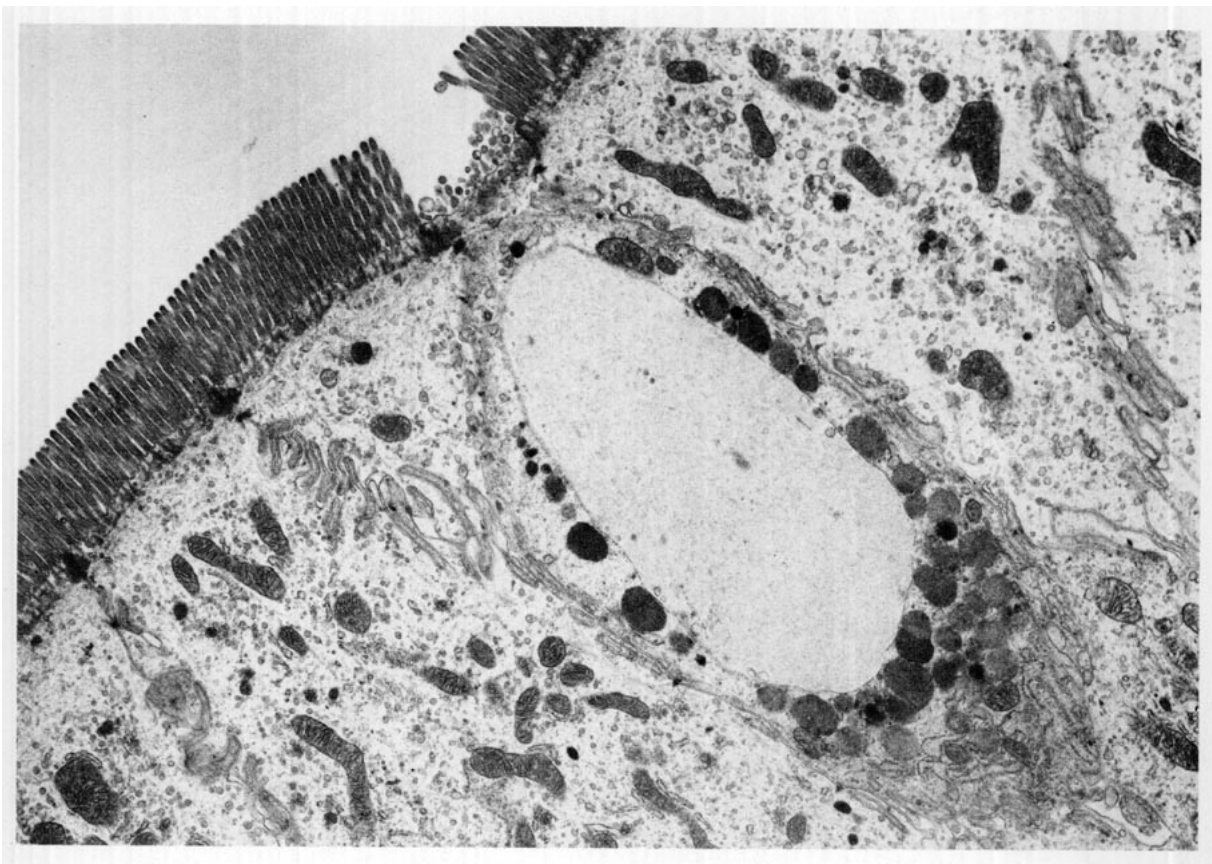

FIGURE 3. Low magnification electron micrograph showing epithelial cells from the surface of one of the villi from the small intestine of a rat. This animal had been maintained on a diet of rat pellets plus butter with which was mixed chrysotile asbestos; 5 mg/g. The cells appear normal and no asbestos penetration has occurred. 14,430×.

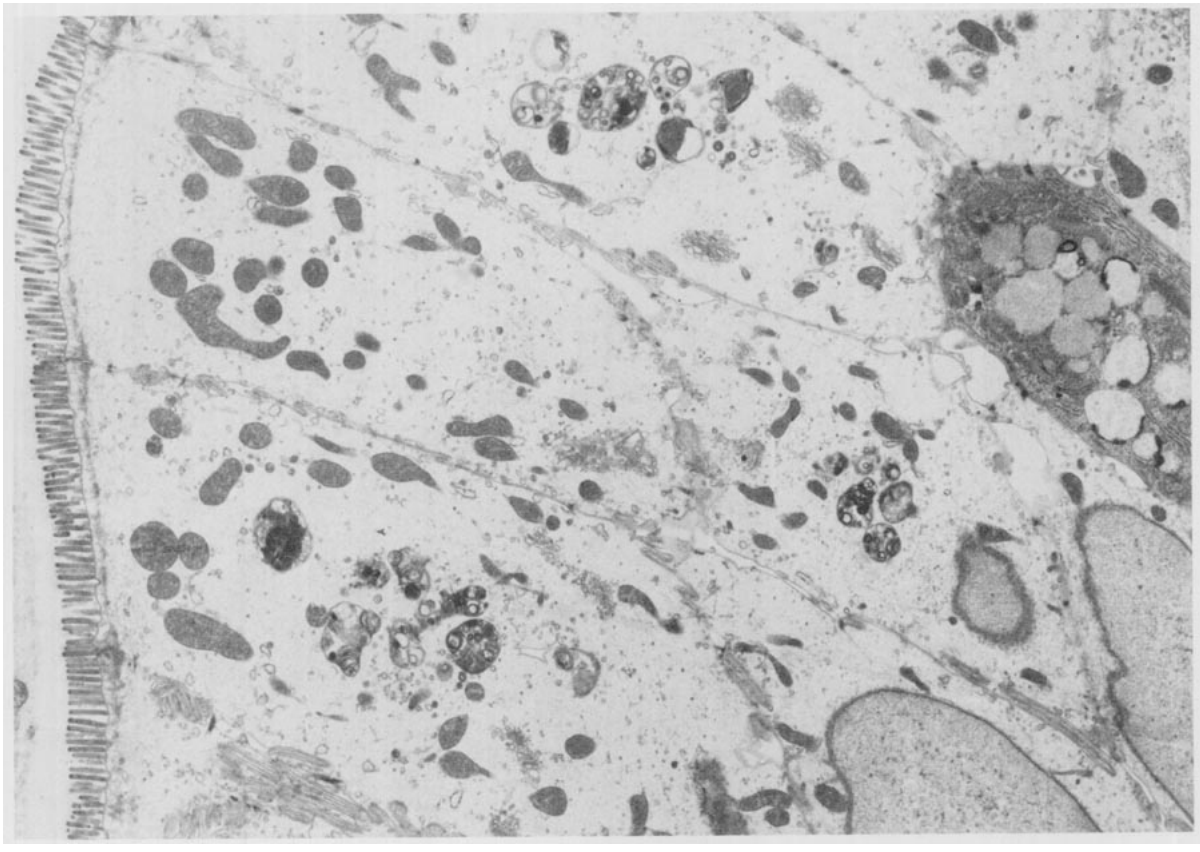

FIGURE 4. Electron micrograph of columnar epithelial cells from the colon of a rat. This animal had been maintained on a diet of rat pellets plus butter containing crocidolite asbestos; 5 mg/g. The cells appear normal and no asbestos penetration can be seen. 9,360×.

penetrating the cell membrane by mechanical action. High magnification photographs (Figs. 5 and 6) indeed show that the problems of mechanical penetration of the surface membrane of gut epithelial cells, may be much greater than with the smooth surface membranes of other cells. Most of the epithelial cells lining the intestine have their surface membranes modified to produce the numerous closely packed microvilli of the brush border. These microvilli are often so close together that even a single chrysotile crystal would be unable to penetrate between them unless they could be separated in some way. We know little about the rigidity of microvilli but it is known that each of these projections contains longitudinal bundles of microfilaments, which project from the base of each microvillus and are combined to form the layer known as the terminal web *(8)*. This combination seems likely to provide quite a formidable barrier to the penetration of large particles. Similar structural considerations make it unlikely that asbestos fibers would be able to penetrate easily between the epithelial cells lining the gut. Whereas some epithelial layers, like the mesothelium, consist of cells which are relatively loosely attached to one another, the columnar epithelial cells of the gut are firmly bound together by numerous desmosomes, and close to the gut surface, the membranes of adjoining cells are closely apposed in membrane junctional complexes.

In summary, our early experimental results suggest that asbestos fibers ingested along with normal quantities of food do not cause damage to the gut linings in rats nor penetrate into the gut epithelial cells. Completed studies will, however, be necessary to be definite about this.

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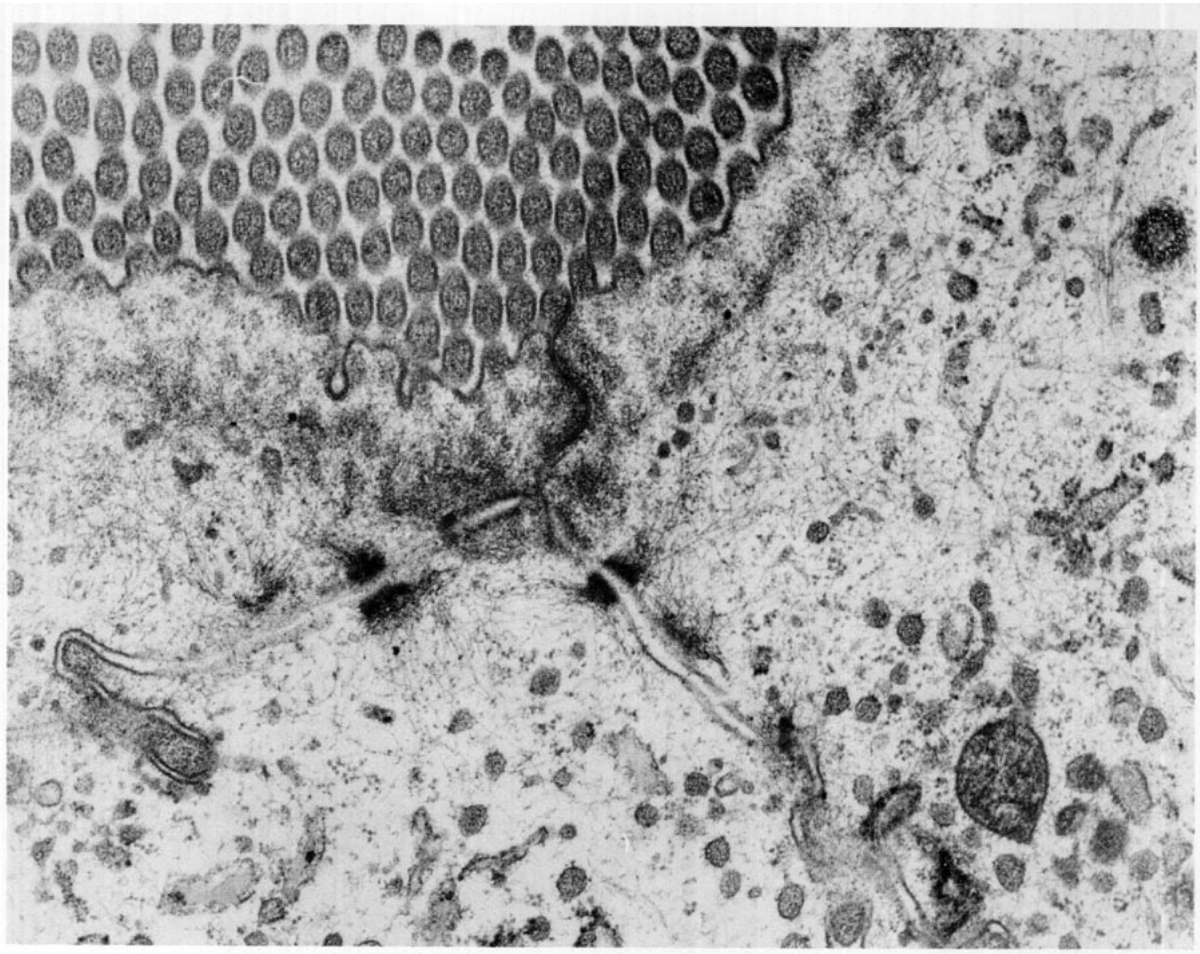

FIGURE 5. High-magnification photograph of cell cytoplasm from the surface of the small intestine in one of the animals fed a butter—asbestos mixture. The cell surface consists of a "brush border" made up of closely packed microvilli and from each microvillus projects a bundle of microfilaments that combine within the cell cytoplasm to form a terminal web. The presence of desmosomes on the cell membranes close to the gut surface indicates that the cells are firmly bound together at this point. 28,470×.

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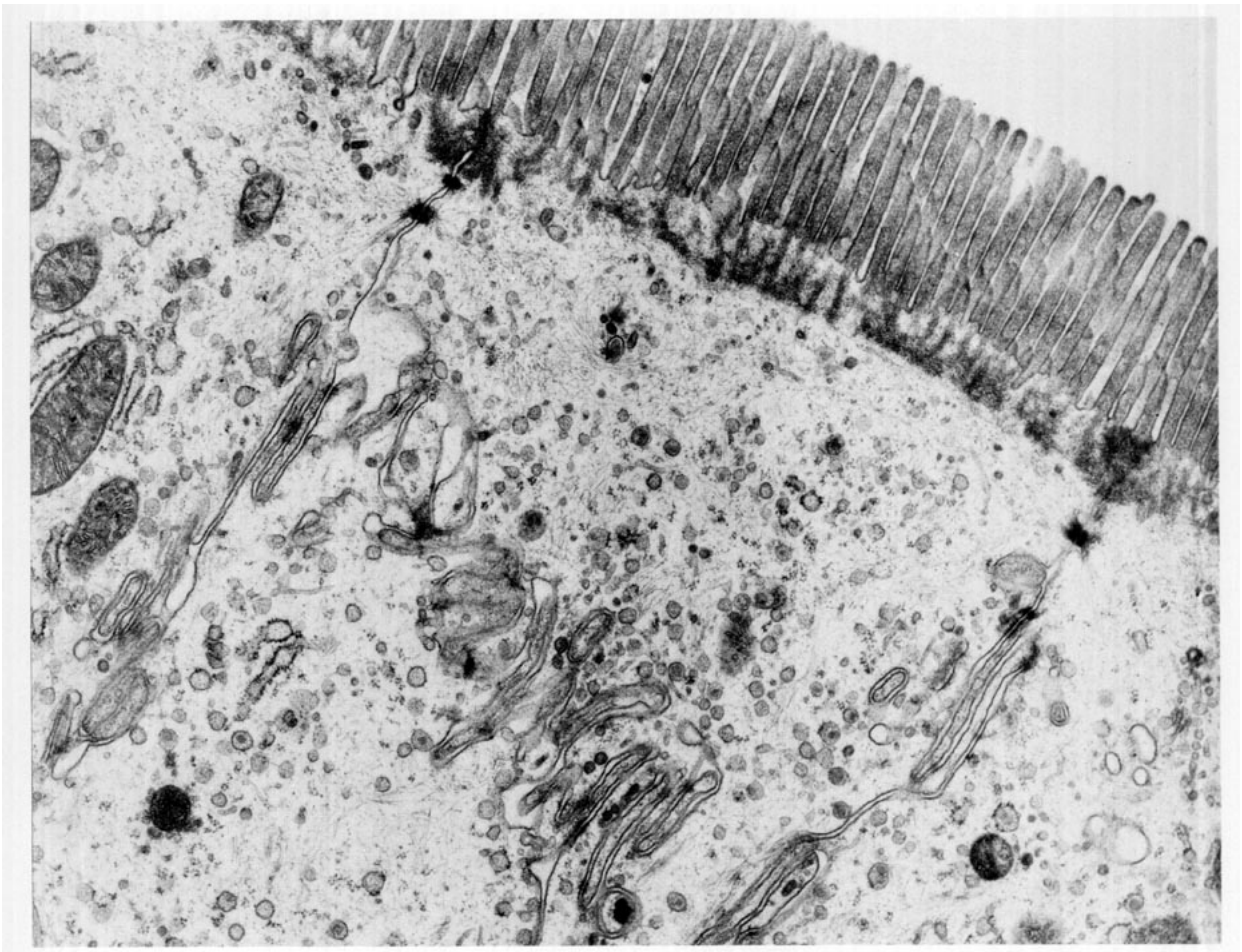

FIGURE 6. Tangential section of a group of epithelial cells from the surface of the small intestine in a rat fed asbestos mixed with butter. Near the gut surface the cells are firmly attached to one another by desmosomes and membrane junctional complexes. The grouping of the transversely cut microvilli shows that these form an unbroken carpet on the gut surface, with no change of pattern to indicate the presence of cell boundaries. 38,220×.